USPIO dynamics in an animal model of multiple sclerosis

R. D. Oude Engberink[1], S. van der Pol[2], H. E. de Vries[2], and E. L. Blezer[1]

[1]Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [2]Molecular Cell Biology and Immunology, VU medical Center, Amsterdam, Netherlands

Introduction

Monocyte infiltration into the central nervous system (CNS) plays an important role in ongoing neuroinflammation and tissue destruction in pathologies like multiple sclerosis and stroke. Earlier research identified monocytes as potential therapeutic target [1] and emphasized the need for a non-invasive tracking method. The development of superparamagnetic particles of iron oxide (SPIO) has made MRI a potential tool for in vivo cell tracking. In studies concerning monocyte infiltration in an animal model for MS (Experimental Autoimmune Encephalomyelitis; EAE), MRI was performed 24h after intravenous (iv) administration of ultra small SPIO (USPIO) [2]. It is believed that USPIO uptake by monocytes occurs in circulation and that hypointense spots on MR images detected after 24h represent actual cell infiltrates. However, it remains unclear what mechanism in vivo is responsible for USPIO entrance into the brain parenchyma. In addition to the infiltration of peripheral labeled monocytes, USPIO might passively diffuse over a ruptured blood brain barrier (BBB) or be transported by activated brain endothelial cells (transcytosis).To identify the fate of USPIO in more detail, the present study performed MR scans on EAE rats directly after USPIO administration. Furthermore, follow-up scans at 24h and 72h were performed on brain, spinal cord and lymph nodes to investigate USPIO persistence in the brain during neuroinflammation.

Material and Methods

Acute EAE was induced in 18 Lewis rats (male, 250g) as described previously [2]. On day 10 (disease onset, n=6) and day 13 (disease peak, n=6), rats were iv administered USPIOs (Sinerem, Guerbet France, 30nm, 17mg Fe/kg). Imaging (4.7T, Varian, Palo Alto, USA, FOV=3.2x3.2cm, matrix= 128x128, 21x 1mm) was performed before and directly after USPIO administration every 30min. up to 6h. Animals were allowed to recover and imaged 24h and 72h later. T_2-relaxation time images were calculated from a multi echo multi slice sequence (TR=3.2s, TE=17.5ms + 9*17.5ms, NEX=4). To assess BBB damage T_1W MRI (TR=300ms; TE=11.5ms, NEX=4) was performed at the end of each scan session before and after Gd-DTPA administration (0.5mmol/kg, 10min in circulation). In addition, 2 healthy rats served as control and received identical USPIO injections. To assess the effect of iron oxide size, a subset of EAE rats (n=2 at disease onset and disease peak) received micron sized particles of iron oxide (MPIO, Bangs Laboratories, 0.96μm, 17mg Fe/ml).

Results

In EAE rats USPIO presence in a lesion was observed within 1h after administration both at disease onset (not shown) and disease peak. The baseline image (before USPIO) showed a hyperintense lesion (Fig. 1, arrowhead) in the brainstem and hypointense spots appeared within 1h after USPIO administration. The overall hypointense area corresponded to areas of Gd-DTPA enhancement at 6h. At disease onset USPIO positive areas were observed at 24h in spinal cord and brainstem (Fig. 2). Interestingly, the hypointense spots were absent again after 72h. Furthermore at 72h, we observed in the animals that received USPIO at disease onset, a hypointense spot at the deep cervical lymph nodes (Fig. 3, arrowhead). USPIO injection at the disease peak showed a similar pattern, but USPIO enhancement was increased at 24h in the cerebellum and hypointensities in the lymph nodes were already observed at 24h. (not shown). USPIO positive areas were absent in brain and deep cervical lymph nodes in healthy control animals. Surprisingly, the MPIO injected animals showed no hypointensities in brain and spinal cord at any MR time point. Lymph node analysis after MPIO injection is currently ongoing.

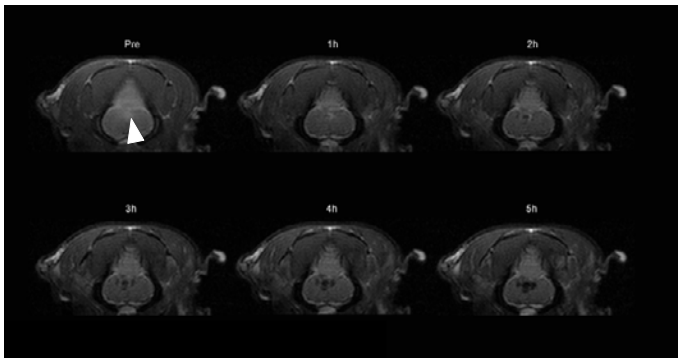


Fig. 1

Fig. 2

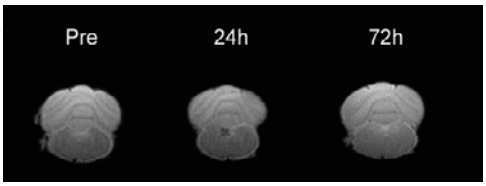
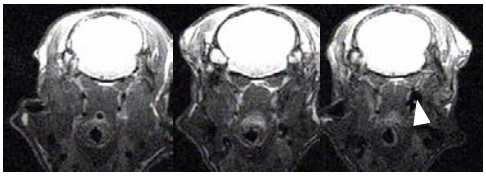


Fig. 3



Discussion

Our results show that USPIO enhancement in brain lesions occurs in an early time frame (within 1h). Most likely in this stage USPIO presence in the CNS is not cell dependent. The absence of MPIO enhancement further supports the idea that particle size and half life in circulation play an important role whether iron oxide particles can cross the BBB. At a later stage the presence at 24h and absence at 72h may reflect cell dependent uptake and migration. The results on cervical lymph node enhancement following USPIO injections trigger further investigation on whether USPIO are extra- or intracellular. In conclusion, this study reports on a novel approach for the use of a single injection of USPIO to identify lesions in an early time frame and to monitor non-invasively the role of the lymphoid system in EAE.

References

[1] Huitinga et al., J Exp Med, 1990

[2] Floris et al., Brain, 2004